Center for Veterinary Biologics and National Veterinary Services Laboratories Testing Protocol

Supplemental Assay Method for Sterility Testing of Live Viral Vaccines of Chicken Embryo Origin Recommended for Nonparenteral Injection

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1. Introduction

1.1 Background

This Supplemental Assay Method (SAM) describes the test procedure used to detect viable bacteria and fungi in live viral vaccines of chicken embryo origin recommended for nonparenteral injection, as prescribed by the Code of Federal Regulations, Title 9 (9 CFR), Part 113.27(e). This test procedure uses Brain Heart Infusion Agar (BHIA) to determine the colony-forming units (CFUs) of contaminating bacteria and fungi.

1.2 Keywords

CEO, poultry vaccines, pour plate

2. Materials

2.1 Equipment/instrumentation

2.1.1 30°-35°C incubator

2.1.2 20°-25°C incubator

2.1.3 Bunsen burner

2.1.4 Biosafety cabinet

2.1.5 Water bath (set to 60°C)

2.2 Reagents/supplies

2.2.1 Individually packaged sterile 1-, 3-, 5-, or 10-cc syringes (size used depends on product volume)

2.2.2 BHIA, see **Section 8.1**

2.2.3 Penicillinase concentrate (Baltimore Biological Laboratory [BBL], Becton Dickinson Microbiology Systems)

2.2.4 Glassware: 500-ml pyrex bottles with orange tops

2.2.5 Sterile water in serum vials: volumes determined by dosage of products to be tested, see **Section 8.2**

2.2.6 Sterile clothes: coveralls, mask, hair bonnet, sleeves, shoe covers, gloves, and protective eyewear

2.2.7 70% ethyl alcohol

2.2.8 Disinfectant

2.2.9 4 x 4 sterile gauze pads

2.2.10 Individually packaged sterile 1-cc pipettes

2.2.11 Petri dishes, 100 x 15 or 150 x 15 mm (as needed)

2.2.12 Vacutainer® needles

3. Preparation for the test

3.1 Personnel qualifications/training

The personnel performing the testing must have experience or training in this SAM. This includes knowledge of aseptic biological laboratory techniques and preparation, proper handling, and disposal of biological agents, reagents, tissue culture samples, and chemicals. The personnel must also have knowledge of safe operating procedures and policies and Quality Assurance (QA) guidelines of the Center for Veterinary Biologics-Laboratory (CVB-L) or equivalent, as well as training in the operation of the necessary laboratory equipment listed in **Section 2.1**.

3.2 Preparation of equipment/instrumentation

3.2.1 Turn on the biosafety cabinets at the beginning of the work wk and leave them on all wk.

3.2.2 Monitor the incubators daily for temperature according to the current version of GDOCSOP0001.

3.2.3 Turn on the water bath approximately 1 hr prior to use. Monitor the temperature to make sure the water is 60°C or less according to the current version of GDOCSOP0002.

3.2.4 Monitor the temperature of freezers and coolers used for the storage of biologicals daily according to the current version of GDOCSOP0003.

3.3 Preparation of reagents/control procedures

3.3.1 Determine the growth-promoting qualities of the BHIA media according to 9 CFR, Part 113.25(b). Use *Bacillus subtilis* and *Candida krusei* as the positive controls for this test procedure. Conduct these positive control tests on each autoclave lot of media according to the most current version of STSAM0902.

3.3.2 Prepare the *B. subtilis* and *C. krusei* reagents according to the current version of the STSAM0900 protocol.

3.3.3 Prepare a working solution of penicillinase by placing 1 ml of the penicillinase stock solution (**Section 2.2.3**) into 99 ml of sterile water.

3.3.4 Technique Controls: Inoculate 2 petri dishes with 0.2 ml each of sterile water from serum vials of the same size as used to rehydrate those biologicals tested. Use all volumes of water used with the tested biologicals for the tech controls. If no water vials were needed with the tested biologicals, then inoculate 2 petri dishes with 0.2 ml each of water from 1 serum vial containing 5 ml of sterile water. Use the same

boxes or lots of syringes used with the tested serials of biologicals to inoculate the sterile water of the tech controls. Pour 20-25 ml of BHIA into each petri dish and swirl. Incubate 1 petri dish from each size of water vial at 30°-35°C for 7 days and 1 petri dish at 20°-25°C for 14 days.

3.3.5 Negative or Media Controls: Incubate 2 representative petri dishes of uninoculated media to confirm the sterility of the autoclaved media (9 CFR, Part 113.25(c). Inoculate 2 petri dishes with 0.2 ml of the 1:100 dilution of penicillinase **(Section 3.3.3)**. Incubate 1 petri dish from each set of media controls at 30°-35°C for 7 days and 1 petri dish at 20°-25°C for 14 days.

3.4 Preparation of the samples

3.4.1 Receive the biological samples to be tested from the Biological Materials Processing Section (BMPS) according to the current version of STSOP0001.

3.4.2 Log in the biological samples by checking the serial numbers of all vials, recording the diluent numbers, assigning a test number, and completing the testing log book (STFRM0271) as stated in the current version of STSOP0011.

3.4.3 Look up the volume of BHIA needed per petri dish for each serial to be tested on the dilution of preservative computer file using the Lotus Approach 97 program and the file name A:\VOLLIST4 as described in the current version of STSOP0020.

3.4.4 Order sufficient BHIA from the media prep department. Add enough extra media to this order to cover positive, negative, and technique controls.

3.4.5 Order sterile purified water in serum vials from the media prep department in sufficient volumes, as determined from the dosage of the product in **Section 8.2**, for those serials without accompanying diluent. Order enough extra water for the technique controls.

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4. Performance of the test

4.1 On the day of the test, wipe off the serials of biologic to be tested with disinfectant. Pay special attention to the cleaning of the tops of the vials and the rubber stoppers.

4.2 Set the wiped-down samples on a tray and then place the tray on a table inside the outer sterility room.

4.3 Early on the day of the test, melt the bottles of BHIA in an autoclave for 30 min at 100°C, then place in a water bath set at 60°C until testing. Check the water temperature before using the BHIA to make sure it has cooled to at least 60°C.

4.4 Gown up for doing the sterility testing by putting on sterile coveralls, booties, sleeves, mask, hair bonnet, gloves, and protective eyewear.

4.5 Wipe down the interior surfaces of the biosafety cabinet used for testing with 70% alcohol.

4.6 Number the petri dishes to coincide with the serials to be tested.

4.7 Add sufficient amounts of the penicillinase working solution (**Section 3.3.3)** to BHIA to yield 500 kinetic (kersey) units of penicillinase/ml of media, just prior to use.

4.8 Place the testing materials (syringes, Vacutainer® needles, 4 x 4 gauze squares, etc.) in the biosafety cabinet or on a cart next to the cabinet.

4.9 Place the samples, petri dishes, and test media for the first serial in the biosafety cabinet.

4.10 Swab the tops of the samples with a 4 x 4 gauze pad soaked in 70% alcohol. Flame the tops of the samples using a Bunsen burner.

4.11 One by one, rehydrate or dilute the 10 vials of the serial, as specified in **Section 8.2**, using a syringe and needle or vacutainer needle. Use the firm's diluent if provided, and sterile water if not.

4.12 Inoculate the appropriate amount (**Section 8.2**) from each vial of the rehydrated and/or diluted product, into each of 2 petri dishes, using a 1-cc syringe. The amount of product inoculated on the petri dishes is determined by the dose/dilution calculation such that 10 doses are placed on each petri dish. This may range from 0.1 ml-0.3 ml. Higher amounts may make the media too soft for plate inversion. Pour the determined amount of BHIA (**Section 3.4.3**) on the petri dishes, swirl, and allow to cool.

4.13 Repeat the procedures in **Sections 4.8 through 4.12** on the other serials of biologic to be tested this day.

4.14 Inoculate the negative controls (**Section 3.3.4**), penicillinase (**Section 3.3.4**), and technique controls (**Section 3.3.3**). Pour 20-25 ml of BHIA agar on these plates, swirl, and let cool.

4.15 Place the petri dishes in the appropriate incubator temperature depending on the volumes of media indicated in **Section 3.4.3** for each incubator temperature. Also place 1 technique, penicillinase, and negative agar control petri dish at each incubation temperature. Incubate those petri dishes placed at 30°-35°C for 7 days and those petri dishes placed at 20°-25°C for 14 days.

4.16 Clean the sterility room by wiping down the interior of the biosafety hood and counter tops with 70% alcohol. Remove paper trash from the sterility room and discard the biological samples (STSOP0001) and any extra media by autoclaving.

5. Interpretation of the test results

5.1 Examine all test vessels incubated at 30°-35°C for colonies on day 7 of the incubation period. Count and record the number of colonies on each petri dish in the 027ST1 log book (STFRM0271). Initial and date the log book where indicated. Make gram stains of all colony types and record these findings in the log book.

5.2 Examine all test vessels incubated at 20°-25°C for colonies, at the end of the test on day 14. Count and record the number of colonies on each petri dish in the 027ST1 log book (STFRM0271). Initial and date the log book

where indicated. Make gram stains of all colony types and record these findings in the log book.

5.3 At 14 days add all colonies (CFU) at each temperature. Divide the CFU at each temperature by 100 (10 plates with 10 doses per plate). This will yield the CFU/dose/incubator temperature. Record these CFU/dose in the 027ST1 log book and on the BMPS test sheet.

5.4 If the average CFU/dose is less than or equal to 1 at both incubation temperatures, the serial is SAT.

5.5 If the average is over 1 CFU/dose at either or both incubation temperatures, order a retest to be conducted, using 20 vials of the same serial, provided, that if the retest is not done the serial or subserial is UNSAT.

5.5.1 When retest samples are received, determine if the diluent serial numbers match the diluent used with the initial test. If these serial numbers match, then test these 20 vials of diluent by 9 CFR, Part 113.26. Conduct the retest on the vaccine vials using sterile water as the diluent.

5.5.2 If the diluent provided with the retest samples is absent or different than that with the initial test, conduct the retest of the vaccine vials with sterile water and discard the diluent.

5.6 If the average retest results are greater than 1 CFU/dose, at either incubator temperature, the serial is UNSAT.

5.7 If the average retest results are less than or equal to 1 CFU/dose, at both incubation temperatures, the serial is SAT.

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6. Report of test results

6.1 Record the test results in CFU/dose and conclusions of SAT or UNSAT in the testing log book and on the BMPS computer test sheet for each serial tested. Initial and date the 027ST1 log book (STFRM0271) and BMPS computer test sheet when taking the test off on the 7th day at 30°-35°C and again on the 14th day at 20°-25°C.

6.2 Enter the results and conclusions recorded on the BMPS test sheet in the CVB-L computer. Receive a computer printout of the result. Check the printout against the BMPS test sheet and log book for accuracy. Check the current version of STSOP0021 for directions on entering test results.

6.3 Forward all test result sheets and computer printouts to the Cytology and Sterility Section (CS) microbiologist or supervisor to check, sign, and date.

6.4 Validate the test results in the computer according to the current version of STSOP0021.

6.5 File the signed and validated test report printouts in the CS files under the first 2 numbers of each serial's product code. File the BMPS test sheet in the same file drawer under the test code number.

7. References

7.1 Code of Federal Regulations, Title 9, Part 113.27(e), U.S. Government Printing Office, Washington, DC, 1999.

7.2 The U.S. Pharmacopeia, 1985, Vol. 21, pp 1151-1160, Mack Publishing Co., Easton, PA.

8. Appendices

8.1 NVSL Media Formula No. 10204

BRAIN HEART INFUSION AGAR (BHIA)

Brain Heart Infusion Agar	52 g
QH_2O	1000 ml

Autoclave 20 min at 121°C.

8.2 Diluent size/dosage and inoculum size/plate

DILUENT SIZE	DOSAGE	INOCULUM SIZE/PLATE
2 ml	100	.2 ml
5 ml	500	.1 ml
10 ml	500	.2 ml
10 ml	1000	.1 ml
15 ml	500	.3 ml
30 ml	1000	.3 ml
50 ml	5000	.1 ml
60 ml	2000	.3 ml
75 ml	2500	.3 ml
100 ml	10000	.1 ml
250 ml	25000	.1 ml
300 ml	10000	.3 ml
80 ml	8000	.1 ml
150 ml	15000	.1 ml